[ ACQUITY UPC² ]

# ULTRAPERFORMANCE CONVERGENCE CHROMATOGRAPHY

Technology that breaks through chiral and achiral challenges

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®


Waters
Acquity UPC²
Convergence Manager
Waters
Acquity UPC²
Sample Manager
POWER
RUN
Waters

# THE ABILITY TO HANDLE CHIRAL AND ACHIRAL SEPARATIONS WITH **UNEQUALED SPEED AND UNPARALLELED CONFIDENCE**

## ACQUITY UPC²

Using inexpensive and non-toxic compressed liquid $CO_2$ as a primary mobile phase, the ACQUITY UPC²® System gives scientists the ability to precisely vary mobile phase strength, pressure, and temperature. With this ability to fine-tune the resolving power and selectivity of the system, scientists can exercise better control over the retention of analytes for separating, detecting, and quantifying structural analogs, isomers, and enantiomeric and diastereomeric mixtures – all compounds that are often a challenge to separate by any other means.

As a member of the ACQUITY® Platform of separations systems, the ACQUITY UPC² System embraces a design concept that emphasizes the functional relationship between the various components, from column chemistries, to software, to the instrumentation itself.

The ACQUITY UPC² System brings the technological advancements of the ACQUITY portfolio to the world of traditional SFC-based separations, combining liquid $CO_2$-based separations with the performance and reliability required by ACQUITY users.

Providing the ability to perform chiral and achiral separations with unequaled speed and unparalleled confidence, pairing ACQUITY UPC² Trefoil™ and Torus™ Column chemistries with the ACQUITY UPC² System gives separation scientists access to the power of normal-phase chromatography in an instrument designed with the ease-of-use and reliability of reversed-phase chromatography in mind.

**Trefoil™ Technology**

**Torus™ Technology**

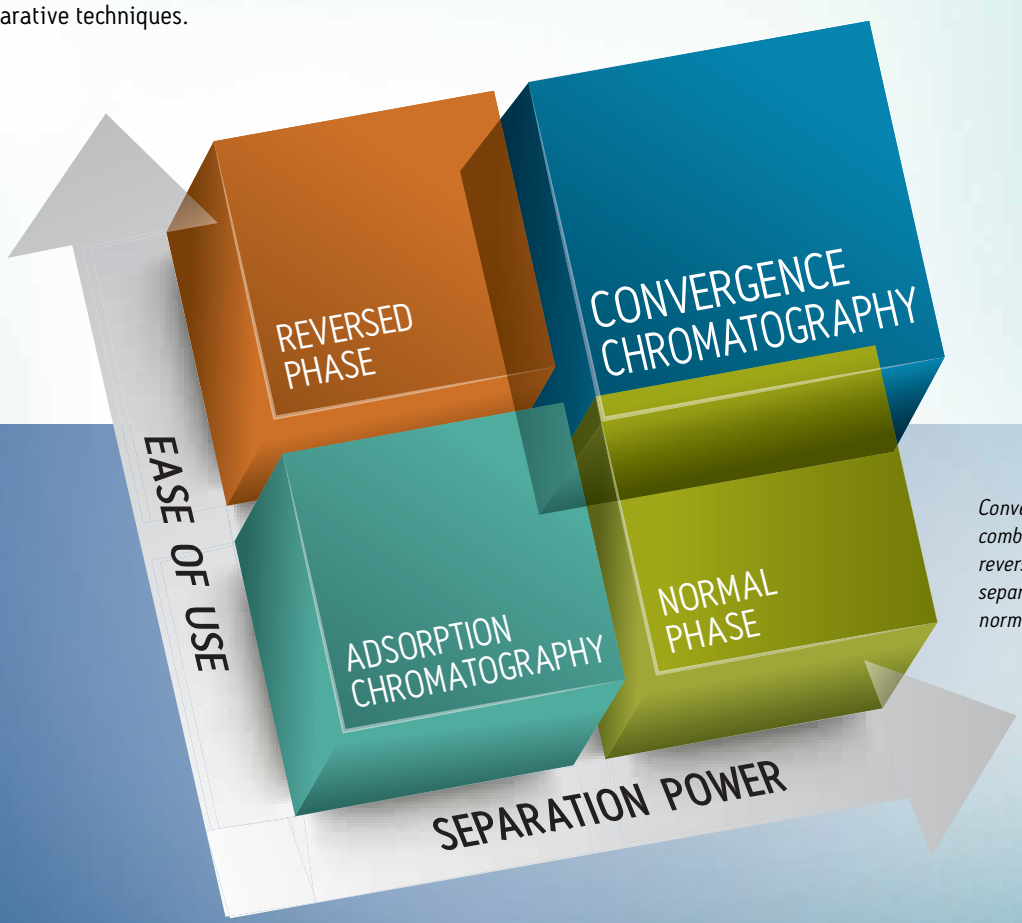
## Convergence Chromatography

In 2012, Waters introduced UltraPerformance Convergence Chromatography™ (UPC$^2$®), a category of separation science that provides an exceptional increase in selectivity to the chromatography laboratory. UPC$^2$ is a holistically-designed chromatographic technology that uses compressed $CO_2$ as the primary mobile phase to leverage the chromatographic principles and selectivity of normal-phase LC while providing the ease-of-use and method development simplicity of reversed-phase LC.

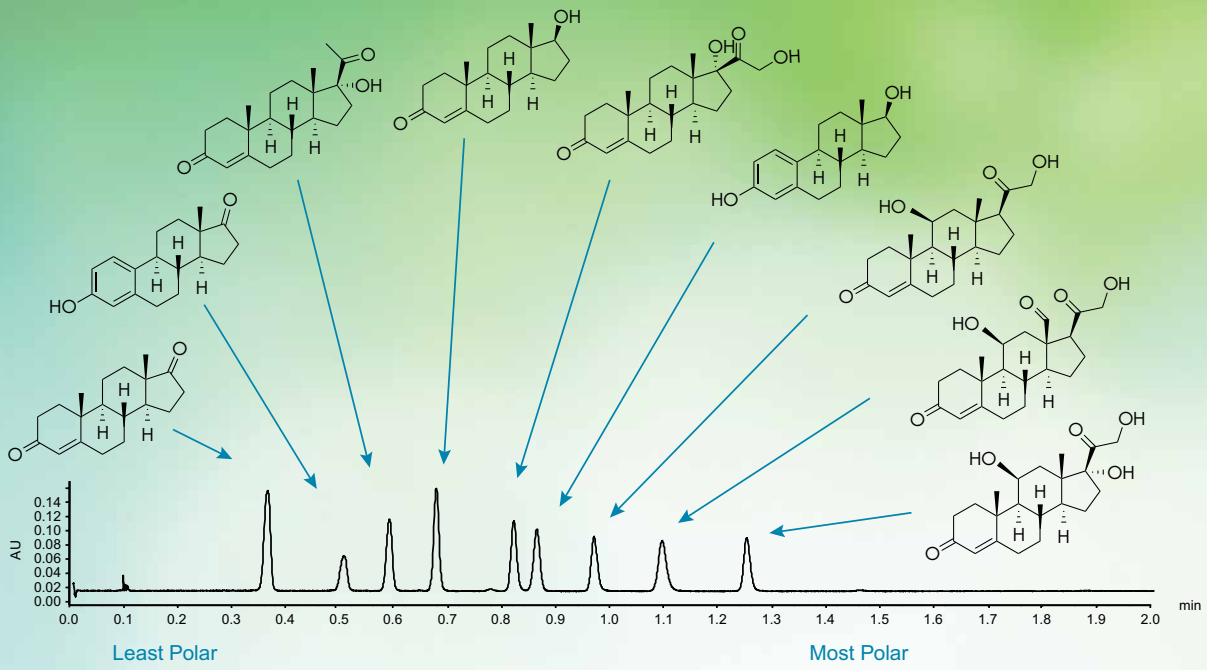
Tracing UPC$^2$ back to its SFC origins, supercritical fluid chromatography exploited density differences of liquefied gases in a supercritical state. This technique has evolved to include the use of co-solvents in a sub-critical state. As it has been demonstrated that liquid $CO_2$-based chromatography can be performed in either supercritical or subcritical states, a new descriptor is required. "Convergence chromatography" is the term used by Waters to cover this modern form of analytical and preparative techniques.

The miscibility of $CO_2$ with a wide range of polar and non-polar organic solvents has made the liquid $CO_2$-based mobile phase versatile enough to separate a much wider range of compounds than reversed-phase chromatography, especially for mixtures containing very hydrophobic and/or polar compounds. Not only can $CO_2$-based mobile phases be used with both polar and non-polar stationary phases, but the chromatography can be influenced by modulating solvent gradients with a much wider choice of columns (including chiral columns) using the same mass spectrometry-compatible co-solvents.

The unique feature of convergence chromatography is not the state or the condition of the solvent, but rather the ability to combine – or converge – the separation of a much wider variety of compounds with one chromatographic system.




*Convergence chromatography combines the ease-of-use of reversed-phase LC with the separation power of normal-phase LC.*



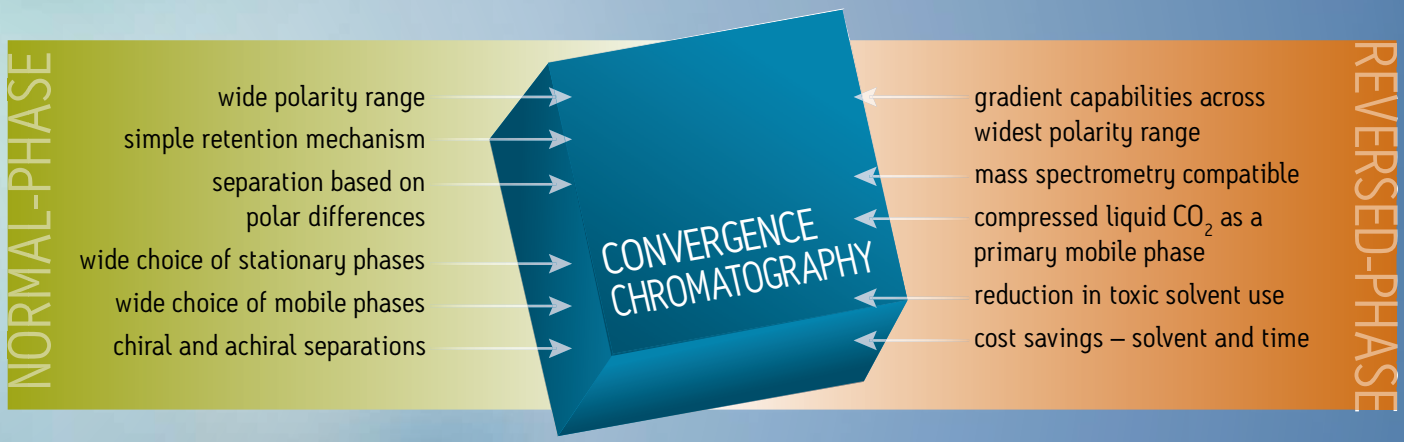

*Convergence chromatography can provide separations according to the overall polarity of the molecules, as shown with a family of closely-related steroids.**

## RETENTION MECHANISMS IN CONVERGENCE CHROMATOGRAPHY

Convergence chromatography has similar retention mechanisms to normal-phase chromatography in that it generally elutes compounds from column stationary phases according to their polarity in the mobile phase. In reversed-phase chromatography, polar compounds are eluted first, often causing separation challenges. In convergence chromatography, however, polar compounds are retained and elute last. In the example chromatogram above, we see that the general elution profile for a range of neutral steroids is from least polar to most polar.

The powerful orthogonal capability of normal-phase separations is elevated to a mainstream technique with the use of compressed liquid $CO_2$ as the primary mobile phase in convergence chromatography. This allows the use of gradients across the widest polarity range and brings full mass spectrometry detection capabilities into everyday laboratory use. The separation of most compounds and mixtures that are soluble in organic solvents is made possible. More than that, compounds that are often a challenge to separate by any other means such as structural analogs, isomers, and enantiomeric and diastereomeric mixtures are now more easily separated using convergence chromatography.

## CONVERGENCE CHROMATOGRAPHY: THE POWER OF NORMAL PHASE **WITH** THE EASE OF REVERSED PHASE




* C. Hudalla *et al.*, Method Development for the Analysis of Endogenous Steroids Using Convergence Chromatography with Mass Spectrometric Detection. Waters Application Note 720004692EN, 2013.

# ACQUITY UPC$^2$ System

### ACQUITY UPC$^2$ PDA Detector (A)

- Photodiode array detector (PDA) has a high-strength silica lens that compensates for differences in refractive index between the $CO_2$ and co-solvents, resulting in significant reduction of baseline noise.
- High-sensitivity, low-volume 10-mm cell accommodates narrow peak widths while maintaining optimal spectral performance, a key requirement for high throughput screening.

### Column Manager (B)

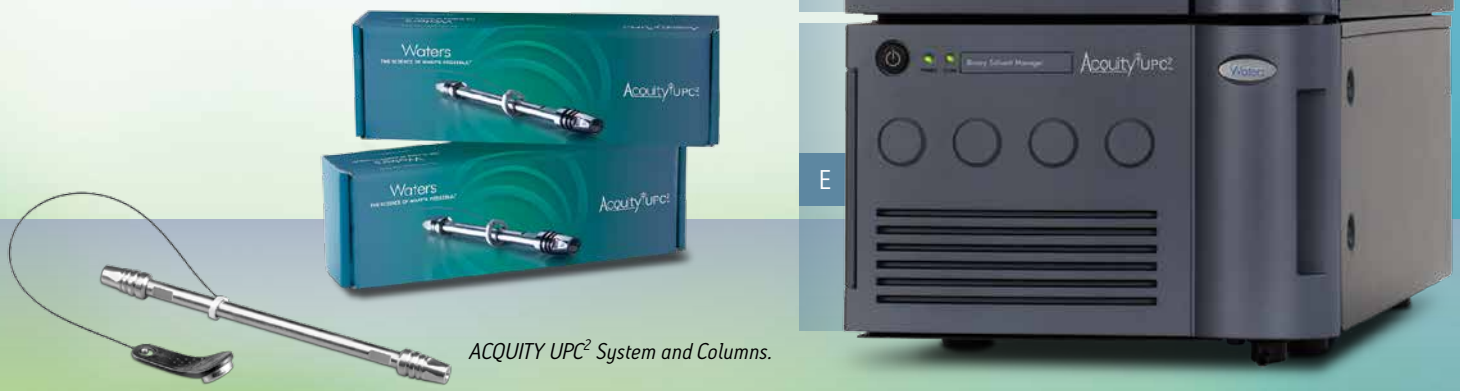
- Advanced thermal control and active solvent pre-heating allow temperature settings in 0.1 °C increments up to 90 °C.
- Multiple column oven options for 2, 4, 6, 8, and 15 columns, allowing a wide range of chiral and achiral columns to be mounted on a single system.

### Convergence Manager (C)

- Two-stage active and static automated back pressure regulator (ABPR) for improved density control.
- This innovative high-frequency, dynamic response ABPR design is critical to the low noise required for ACQUITY UPC$^2$ System performance.

### Sample Manager (D)

- Partial loop injector technology as standard.
- Accurate, precise, reproducible; 0.1–50 µL in 0.1-µL increments.
- Exceptionally low carryover: (<0.005%) and injector linearity: (>0.999 $R^2$) made possible by utilizing ACQUITY Technology.




*ACQUITY UPC$^2$ System and Columns.*

## Binary Solvent Manager (E)

The heart of the ACQUITY UPC² System is defined by its unique pumping capabilities. Separate pumping systems are used for metering the liquid $CO_2$ and the co-solvents.

Although both pumping systems are UPLC® pumps, one is modified specifically for liquid $CO_2$ and features two-stage Peltier cooling.

The ability to accurately meter compressible liquid $CO_2$ to the same accuracy as noncompressible co-solvents prior to mixing is critical to performance and robustness.

This is most clearly seen by the ACQUITY UPC² System's ability to perform <5% co-solvent in 0.1%-increments, starting from 100% $CO_2$. This addresses the most common shortfall in previous generation systems, and is central to the system's robust, reliable reputation.

## Method Development Column Manager

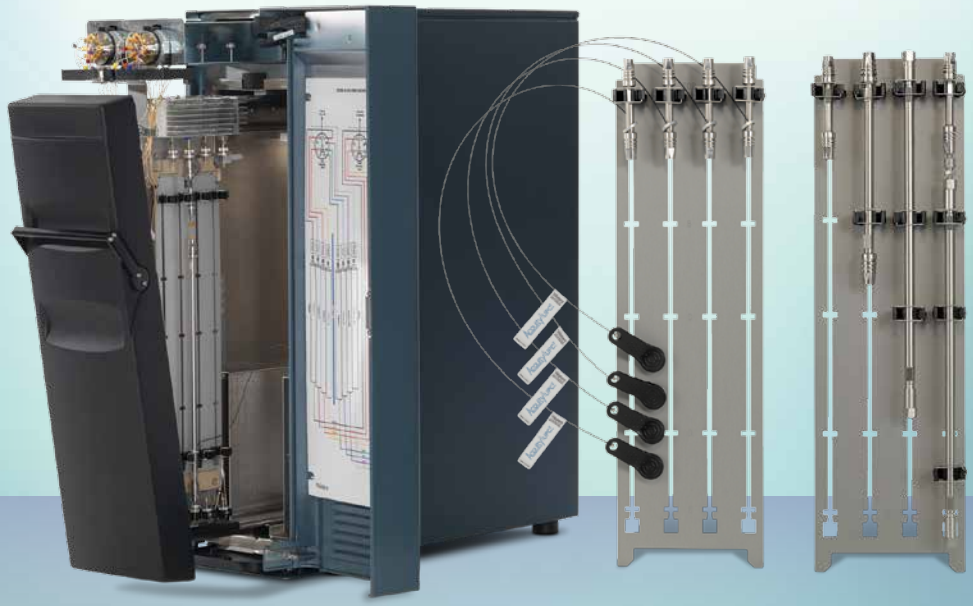
Adding the easy-to-install ACQUITY 30-cm Single-Zone Column Manager (CM-30S) increases productivity due to its capacity to hold up to eight columns ranging in length from 50 mm up to 300 mm, and with internal diameters ranging from 2.1 mm up to 8.0 mm.

The ability to screen multiple column chemistries, modifier proportions, back-pressure settings, and flow rates for a range of different column dimensions creates a flexible method development platform suitable for chiral and achiral analysis on a single system.

Under MassLynx® Software control, the single, 8-column oven can be used for fast screening prior to preparative scale-up, or in open-access mass-directed assays. Under Empower® Software control, a second oven can be used in tandem, allowing control of 15 columns.

Column holding plates on each side of the sliding draw are capable of holding columns ranging in length from 50 mm up to 300 mm. The holding plates are easily installable and removable with the simple action of a lever arm – facilitating faster method development.

With the addition of passive pre-heaters connected to each column position, refractive index effects are mitigated, as the solutes temperature is held at equilibrium equally for all eight columns.



*ACQUITY CM-30S and column holding plates.*

# Convergence Chromatography System Configurations with Mass Spectrometry

## ACQUITY UPC$^2$ WITH ACQUITY QDa DETECTOR

The standard 8/15 column screening system using PDA detection is being used in both chiral and achiral analysis labs. Adding the Waters ACQUITY QDa® Detector and ACQUITY Isocratic Solvent Manager allows for a much higher level of confidence with mass spec-based tracking and troubleshooting, making method development more efficient.

With the significantly increased levels of orthogonality achieved with convergence chromatography, this mass detection-based system designed for routine use is becoming an essential everyday tool for chiral and achiral analysis.

*ACQUITY UPC$^2$ System with ACQUITY QDa Detector.*

## ACQUITY UPC$^2$ WITH XEVO TQ-S

Analysis within the realm of bioanalysis/DMPK requires the highest levels of resolution, sensitivity, and efficiency. The addition of ACQUITY UPC$^2$ Technology to the DMPK lab can address many areas of concern for reversed-phase DMPK separations, such as:

- polar compound or metabolite retention
- fast generic gradient chiral methods
- chiral metabolite separations
- orthogonal separation analysis to reversed phase, either for compounds of interest or matrix interferences

*ACQUITY UPC$^2$ System with Xevo® TQ-S.*

## ACQUITY UPC² WITH TRIPLE DETECTION

Extended capability of a system that offers the most in screening and detection comes in the form of the ACQUITY UPC² System with triple detection.

This method development tool provides maximum column and solvent screening while also providing the best in detection capability. When the analysis challenge includes compounds of interest with no UV chromophore or ionizable groups, the ability to add a third, evaporative light scattering detector to the system can be critical in ensuring that nothing is overlooked.

*ACQUITY UPC² System with ACQUITY UPLC® ELS, ACQUITY UPC² PDA, and ACQUITY QDa detectors.*

## ACQUITY UPC² WITH XEVO G2-XS

The use of an ACQUITY UPC² System with QTof technology provides scientists with the tools to address complicated analysis areas such as lipids or natural products.

Taking advantage of the wider separation of convergence chromatography, polar and non-polar compound classes such as lipids can be addressed with a simple switch of column and gradient conditions to provide accurate mass with high resolution. A system solution designed to:

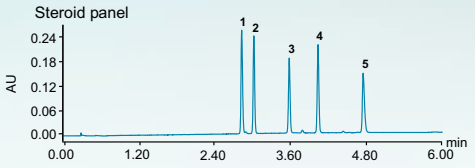
- identify
- quantify
- confirm

*ACQUITY UPC² System with Xevo G2-XS.*

## ENHANCED RESOLUTION OF STEROIDS, VITAMIN A, AND VITAMIN E

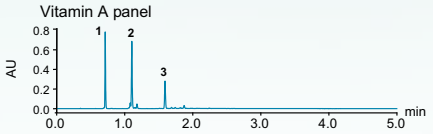
The ACQUITY UPC$^2$ System harnesses the power of SFC to assist in the separation of complex hydrophobic samples, such as steroid and fat-soluble vitamin samples.

Using ACQUITY UPC$^2$ Torus 1-AA Columns, an analyst can rapidly perform the analysis of these challenging compound classes where low % co-solvent use (<5%) is necessary.



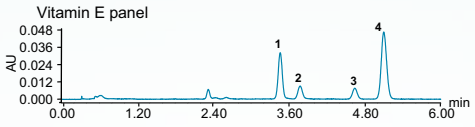

**Compounds:**
1. 17α-Hydroxy-progesterone
2. Estrone
3. Cortisol
4. Estradiol
5. Corticosterone

Column: ACQUITY UPC$^2$ Torus 1-AA, 1.7 µm, 3.0 x 100 mm (p/n 186007629)
Gradient: 3–30% methanol
Flow rate: 1.5 mL/min
Temp.: 40 °C
ABPR: 1,800 psi
UV detection: 220 nm




**Compounds:**
1. Retinyl acetate
2. Retinol palmitate
3. Retinol

Column: ACQUITY UPC$^2$ Torus 1-AA, 1.7 µm, 3.0 x 100 mm (p/n 186007629)
Gradient: 3–40% methanol
Flow rate: 1.5 mL/min
Temp.: 35 °C
ABPR: 1,500 psi
UV detection: 310 nm



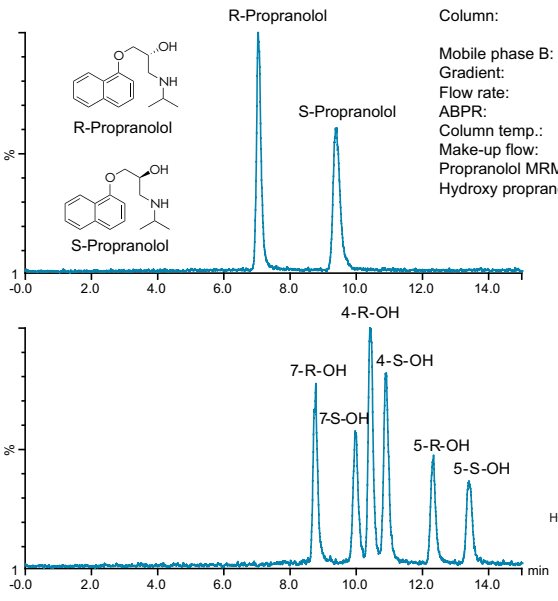

**Compounds:**
1. α/β/γ-Tocopherol
2. δ-Tocopherol
3. γ-Tocotrienol
4. δ-Tocotrienol

Column: ACQUITY UPC$^2$ Torus 1-AA, 1.7 µm, 3.0 x 100 mm (p/n 186007629)
Isocratic: 3% methanol
Flow rate: 1.5 mL/min
Temp.: 35 °C
ABPR: 1,500 psi
UV detection: 290 nm

*Steroid and vitamin panels using ACQUITY UPC$^2$ Torus 1-AA Column.*

## DMPK ENANTIOMERIC SEPARATIONS OF DRUGS AND METABOLITES USING UPC$^2$-MS/MS

Many drug candidates, as well as their metabolites, contain one or more chiral centers. The ability to identify and monitor the various chiral forms of a compound and its metabolites is an essential step during the drug development process, and is easily achieved using convergence chromatography. The combination of ACQUITY UPC$^2$ Trefoil CEL1 Columns with mass spectrometry detection facilitated the easy enantiomeric separation and detection of parent compound propranolol and its three hydroxy metabolites 4-hydroxypropranolol, 5- hydroxypropranolol, and 7-hydroxypropranolol in under 15 minutes.




Column: ACQUITY UPC$^2$ Trefoil CEL1, 2.5 µm, 3.0 x 150 mm (p/n 186007464)
Mobile phase B: 20 mM ammonium formate
Gradient: 5 to 16 over 15 min
Flow rate: 2 mL/min
ABPR: 3,250 psi
Column temp.: 30 °C
Make-up flow: Methanol at 0.25 mL/min
Propranolol MRM: 260 > 183
Hydroxy propranolol MRM: 276 > 173

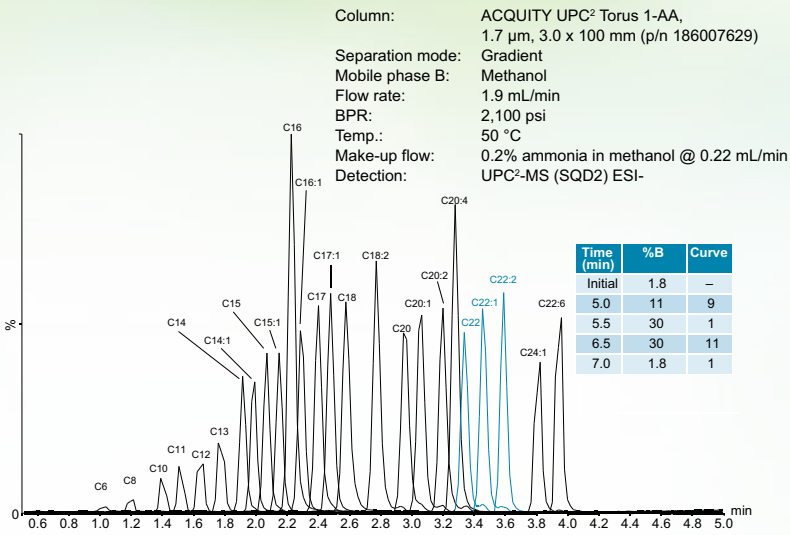
*Separation of propranolol and hydroxy metabolites. Note that each hydroxy metabolite has an R and S form.*

## FREE FATTY ACID LIPID SEPARATIONS WITHOUT DERIVATIZATION

The elution order of free fatty acid (FFA) species depends on the length and the number of double bonds on the fatty acid chain. In a typical reversed-phase LC separation, the longer and the more saturated the acyl chain, the longer the retention time.

In a separation using an ACQUITY UPC$^2$ Torus 1-AA Column, increasing degrees of unsaturation increases the retention time of the FFA. This reduces the number of co-eluting lipid species in complex biological samples containing saturated and unsaturated FFA species with different carbon chain lengths, resulting in a simplified analysis.

In addition, the organic layer extract containing the lipids can be injected directly into the system, omitting the need for solvent exchange for compatibility with reversed-phase LC methods.




*The ACQUITY UPC$^2$-MS FFA analysis provides a simple and fast method with a significant reduction in analysis time compared to alternative techniques such as GC-MS, which requires FAME derivatization.*

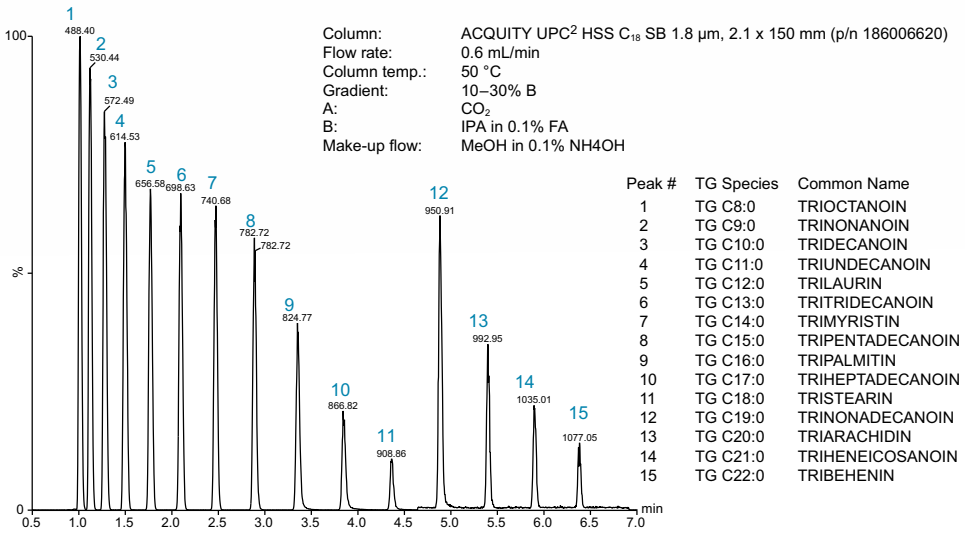
## LIPID ANALYSIS

Multiple types of lipids have been analyzed by ACQUITY UPC$^2$:

- Prostaglandins
- Eicosanoids
- Acylglycerols, mono-, di-, and tri-
- Cholesterol esters
- Sphingolipids
- Sugar isomers
- Phospholipids

And in many matrices:

- Tissues (adipose)
- Whole blood
- Oil algae extracts
- Cow milk
- Edible oils
- Biodiesel




*Analysis of intact triacylglycerol using UPC$^2$-MS containing a complex mixtures of 15 saturated TAG standards (Nu-Check GLC 768). Typical chromatographic methods for the analysis of TAGs are RP-HPLC and GC-MS. RP-HPLC analysis of TAGs take long chromatographic times (2 to 3 hours) and require the dry down and reconstitution of extracts in an RP-compatible injection solvent. GC-MS analysis of TAGs requires a labor intensive FAME derivatization step.*

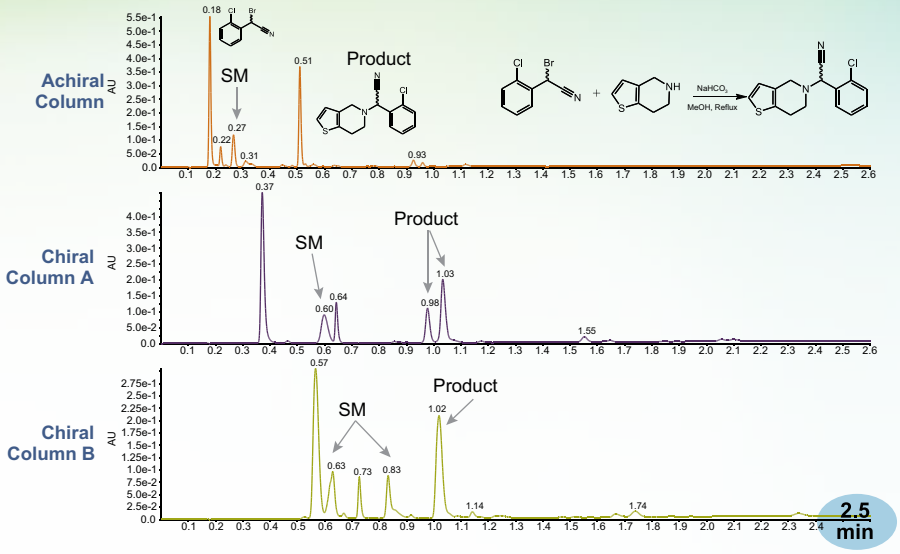
## SYNTHETIC CHEMISTRY – CHIRAL AND ACHIRAL

In synthetic chemistry/medicinal chemistry labs, ACQUITY UPC$^2$ is being used in support of both chiral and achiral analyses.

Whether it is used for achiral synthetic reaction monitoring and optimization, or for chiral enantiomeric excess (ee) conversion monitoring, the same system and eluents can often be utilized.

Having the ability to host eight columns in a single column oven enables open-access use for a wide range of columns and mobile phases. The columns can stay permanently mounted while the user can simply switch between a wide choice of chiral and achiral phases for simplified method development.

It is also possible to switch between short, fast method scouting dimensions and longer method optimization columns with simple software control.



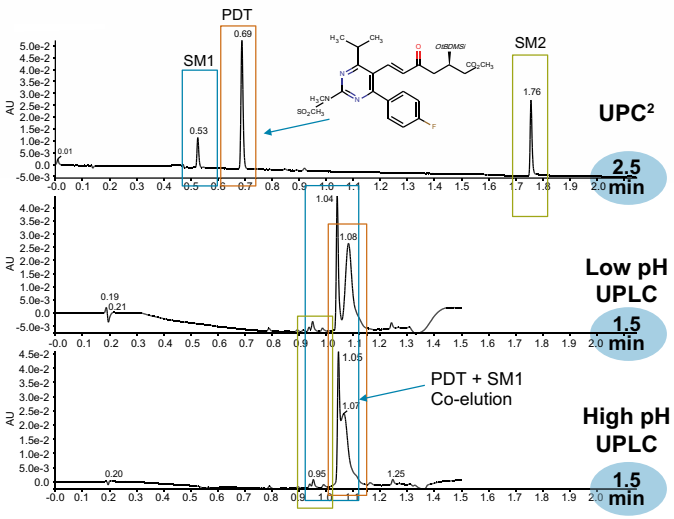

*With ACQUITY UPC², both chiral and achiral separations can be accomplished with the same eluent and without swapping instruments. Shown here, the starting material and the product in this particular reaction step require different chiral columns to resolve their enantiomers.*

## SYNTHETIC CHEMISTRY – ORTHOGONAL TO REVERSED PHASE

In synthetic chemistry/medicinal chemistry labs, ACQUITY UPC$^2$ is being used in rapid screening methods as a clearly viable addition to reversed-phase systems, whether it be in central support labs or in open-access environments. UPC$^2$ is rapidly gaining acceptance because of its ability to separate a wider polarity range in an orthogonal separation mode using simple generic gradient methods that can be easily connected to mass spectrometry.

ACQUITY UPC$^2$ is particularly useful in labs where the separation challenge involves structurally similar compounds, very polar or very non-polar compounds, or when simple orthogonality from reversed-phase separations is desired.

Often, what is difficult by reversed-phase LC is simple by ACQUITY UPC$^2$: chiral and achiral analysis on the same system, using common solvents and additives, all with permanently mounted column banks to simplify the user experience.

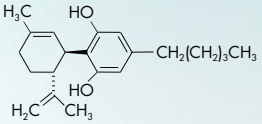


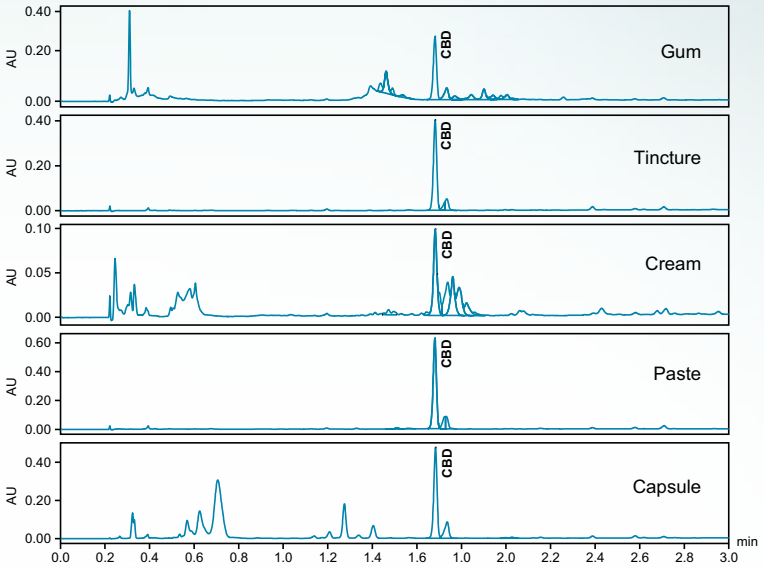

*With ACQUITY UPC², orthogonality to the most commonly used high/low pH approach in reversed phase can be clearly seen as a useful addition to the separation challenges faced by synthesis analysis.*

## RAPID QUANTITATIVE ANALYSIS OF CANNABIDIOL (CBD) FROM 5 CONSUMER PRODUCT FORMULATIONS

Cannabidiolic acid (CBDA) is produced in large abundance in some therapeutic hemp cultivars. Cannabidiol (CBD), is the heat induced decarboxylation product of CBDA and is non-psychoactive and thought to have a wide scope of potential medicinal benefits. CBD has traditionally been administered by smoking or vaporizing (thereby converting CBDA to CBD), however alternative formulations (e.g., topical creams) are now widely available. The therapeutic hemp is processed to ensure that any CBDA is present in these products as CBD. Separation of CBD from excipient materials was achieved in 3 minutes per sample and proved suitable for quantitation. This methodology is suitable for laboratories performing quality control or product quality monitoring of CBD content with a wide range of product formulations.

| | |
|---|---|
| System: | ACQUITY UPC² with PDA Detector |
| Column: | ACQUITY UPC² Torus 2-PIC, 1.7 µm, 3.0 x 100 mm (p/n 186007602) |
| Mobile phase A: | $CO_2$ |
| Mobile phase B: | Methanol |
| Flow rate: | 2.0 mL/min |
| Gradient: | 3–15% B over 3 minutes |
| Column temp.: | 50 °C |
| ABPR: | 1800 psi |
| UV detection: | 228 nm (compensated 500–600 nm) |
| Injection volume: | 1.0 µL |



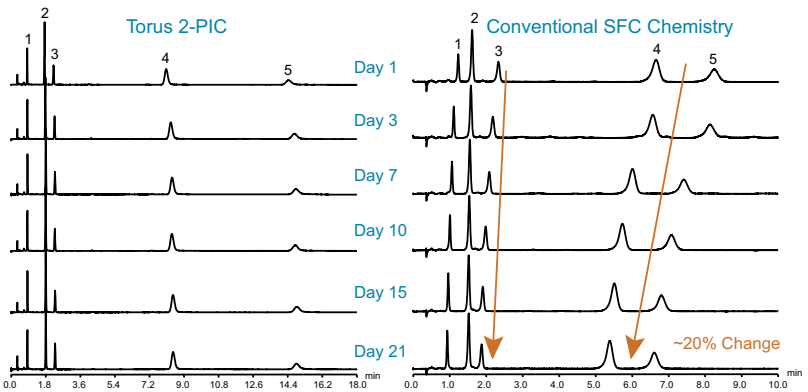

*Cannabidiol (CBD) analysis from five different formulations.*

## INCREASED ROBUSTNESS WITH ELIMINATION OF RETENTION DRIFT

One common concern with silica-based SFC columns is changes in retention while in use. Studies have shown that both selectivities and retention times may shift over time.* In some cases, the column may need to be regenerated in order to establish original performance. ACQUITY UPC² Torus Columns are designed to eliminate changes in selectivity and retention, leading to greater method robustness and extended column lifetimes.

| | |
|---|---|
| Column: | 3.0 x 100 mm |
| Flow rate: | 1.5 mL/min |
| Isocratic mobile phase: | 90% $CO_2$/10% MeOH |
| Temperature: | 35 °C |
| ABPR: | 2500 psi |
| UV detection: | 220 nm |

**Compounds:**
1. Papaverine
2. Fenoprofen
3. Prednisone
4. Sulfamethoxazole
5. Sulfanilamide




*ACQUITY UPC² Torus Columns can benefit both method development and QC scientists with the added performance of maintaining retention and selectivity over time.*

* K. Ebinger, H.N. Weller, *J. Chromatogr. A*, Comparative assessment of achiral stationary phases for high-throughput analysis in supercritical fluid chromatography. 2014 http://dx.doi.org/10.1016/j.chroma.2014.01.060

# ACQUITY UPC² Trefoil Columns

**The best choice for fast, robust chiral separations**

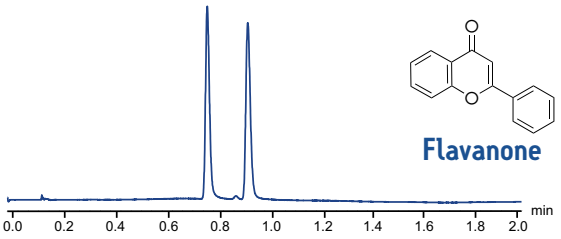
- Optimized particle size, column dimension, and flow rates
- Takes full advantage of mass spectrometry detection
- Faster results with new method development protocols
- High quality, consistent, and reproducible columns

Trefoil™ Technology

ACQUITY UPC² Trefoil Columns are uniquely designed for the ACQUITY UPC² System to enable both selectivity and speed in chiral separations and to reduce method development time. Trefoil Technology Columns are based on modified polysaccharide-based stationary phases for broad-spectrum chiral selectivity.

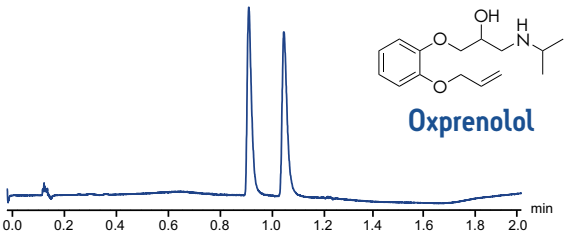
**ACQUITY UPC² Trefoil AMY1, 2.5 µm Columns**

Amylose tris-(3,5-dimethylphenylcarbamate)



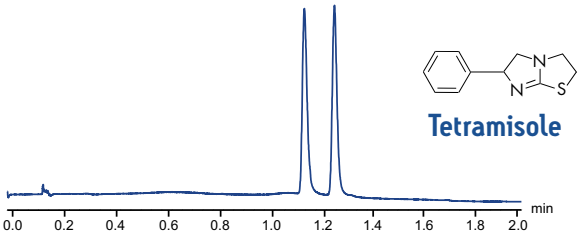

**ACQUITY UPC² Trefoil CEL1, 2.5 µm Columns**

Cellulose tris-(3,5-dimethylphenylcarbamate)




**ACQUITY UPC² Trefoil CEL2, 2.5 µm Columns**

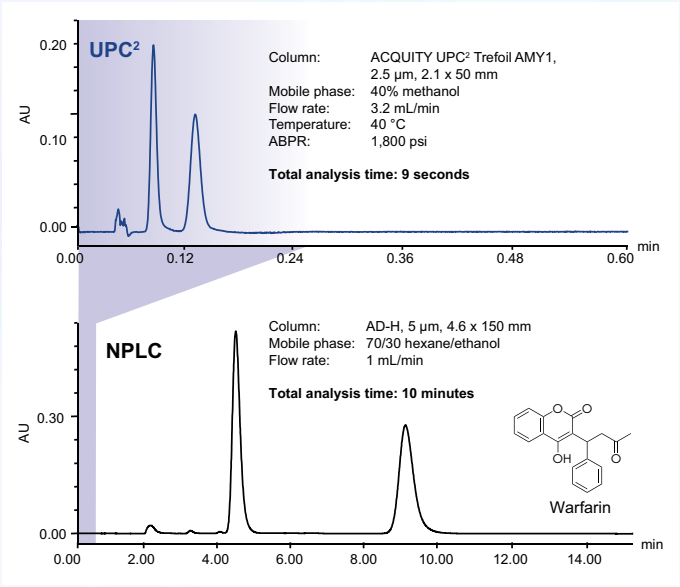
Cellulose tris-(3-chloro-4-methylphenylcarbamate)




*Chiral separations were all run using the 2-minute screening method.*

## TRANSFER OF NORMAL-PHASE CHIRAL TO CONVERGENCE CHIRAL METHODS

Legacy normal-phase (NPLC) chiral methods can be easily transferred to the ACQUITY UPC² System using ACQUITY UPC² Trefoil Columns. Many of these old methods have undesirable characteristics such as long run times and often use chlorinated solvents in combination with THF or hexane, which are costly to purchase and dispose. With simple redevelopment, new cost-effective methods can be obtained using inexpensive and non-toxic compressed liquid $CO_2$ as the primary mobile phase, and can be coupled to mass spectrometers for greater information.



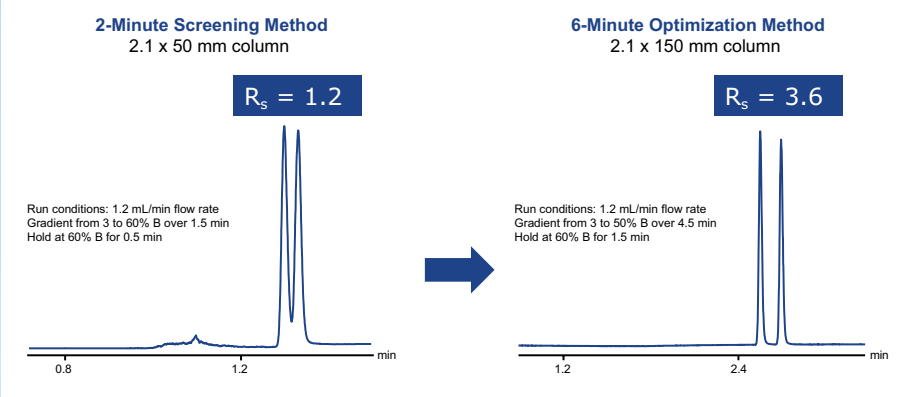

*UPC² can be 30x faster, use 75x less solvent per run, and with 100x lower cost per analysis..*

## NEW CHIRAL METHODS USING CONVERGENCE CHROMATOGRAPHY USING ACQUITY UPC²

Faster method development is possible when combining the dependable, high-performance, low-dispersion analytical ACQUITY UPC² System with the Trefoil Technology stationary phases. Using short, narrow-bore columns with a small number of well-selected co-solvents and mass spectrometry-compatible additives enables this holistic combination to achieve routine gradient screening runs in two minutes.

High-efficiency, narrow-bore columns use 46% less solvent than traditional 4.6-mm columns. Increased sensitivity, reduced solvent use, and faster methods while using mass spectrometry detection offers confidence in confirmation.

Method development scientists seek faster approaches to achieve their desired chiral separations in the shortest amount of time and with the fewest number of method screening injections. To facilitate the search, Waters performed an experimental study using 55 diverse racemic compounds on the ACQUITY UPC² System with ACQUITY UPC² Trefoil Columns. In this study, 44 different blends of co-solvents and mass spectrometry-compatible additives were examined to determine which blends most favorably modulated chiral recognition. This allowed Waters to recommend a method development screen to achieve the highest enantiomer separation success rate with the least number of steps on the ACQUITY UPC² Trefoil Columns.




*An example of the increased resolution expected when moving from the two-minute screening method to the six-minute optimization method.*

The study data analysis for the three chiral stationary phases (CSPs) – ACQUITY UPC² Trefoil AMY1, CEL1, and CEL2 – showed that 44 out of 55 compounds (80%) were resolvable. Of those resolvable compounds, 96% of them could be separated using just four runs via optimal pairing of the blends and the ACQUITY UPC² Trefoil Columns. Using single solvents instead of optimal blends separated only 82% of these compounds. This demonstrates the advantage of using this optimal path screen with the blends of solvents and additives given in the table below.

| STEP | COLUMNS AND BLENDS | CUMULATIVE % SUCCESS |
|---|---|---|
| 1 | AMY1<br>Ethanol/Isopropanol/Acetonitrile<br>Ammonium Acetate | 46% |
| 2 | CEL1<br>Methanol/Isopropanol<br>Trifluoroacetic Acid | 73% |
| 3 | CEL2<br>Ethanol/Acetonitrile<br>Trifluoroacetic Acid | 86% |
| 4 | AMY1<br>Ethanol/Isopropanol<br>Trifluoroacetic Acid | 96% |

**2-Minute Screening Method**
ACQUITY UPC² Trefoil 2.1 x 50 mm Columns
1.2 mL/min flow rate

**2.5 min cycle time per step**

Routine method development for chiral compounds is therefore possible within 10 minutes using the three CSP's and four blended gradient runs. The method development strategy and the study that led to it is made possible using ballistic 2-minute gradient runs with short, small I.D. columns containing the efficient 2.5-µm particle size Trefoil Technology chemistries, which are optimized for convergence chromatography.

Torus™ Technology

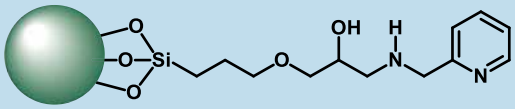
# ACQUITY UPC² Torus Columns

**A family of achiral chemistries designed to set a new performance standard**

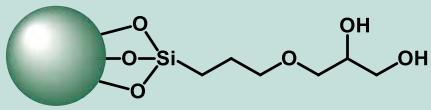
- Excellent peak shapes, with or without additives
- Wide range of unique selectivities with unique ligands
- Highest efficiency and QC-ready robustness
- Modified ligand designed for lipids and fat-soluble vitamins

ACQUITY UPC² Torus Columns are specifically designed to use the complete range of capabilities of the ACQUITY UPC² System to achieve fast, robust achiral separations. ACQUITY UPC² Torus Columns simplify the method development process with four completely new and innovative 1.7-µm chemistries for convergence chromatography. These columns are designed for excellent peak shape that eliminate or reduce the need for additives, and offer added selectivity for a wide range of compounds and improved robustness.

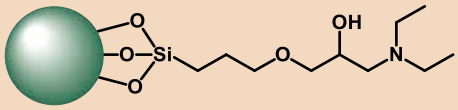
**ACQUITY UPC² Torus 2-PIC, 1.7 µm Columns**
2-Picolylamine



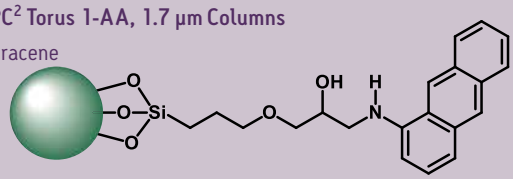

**ACQUITY UPC² Torus DIOL, 1.7 µm Columns**
High Density DIOL




**ACQUITY UPC² Torus DEA, 1.7 µm Columns**
Diethylamine




**ACQUITY UPC² Torus 1-AA, 1.7 µm Columns**
1-Aminoanthracene




The Torus phases are based on a new patent-pending two-stage functionalization of ethylene bridged hybrid (BEH) particles. Modification of the stationary-phase surface during traditional SFC separations has been identified as a primary source of chromatographic variation. The ACQUITY UPC² Torus family of columns addresses this issue through a two-stage bonding process which protects the stationary-phase surface from these undesired reactions, resulting in chromatographically robust columns. The initial bonding provides a hydrophilic surface that controls the retention characteristics of the sorbent, and is responsible for minimizing unwanted surface interactions, which lead to retention and selectivity changes over time. The second step of the functionalization is responsible for the individual selectivity and peak shape characteristics of each of the Torus chemistries. The results of these steps are a series of stationary phases with broad ranging selectivities, which maintain robust chromatographic performance over the lifetime of the column.

# Achiral Torus UPC² Method Development

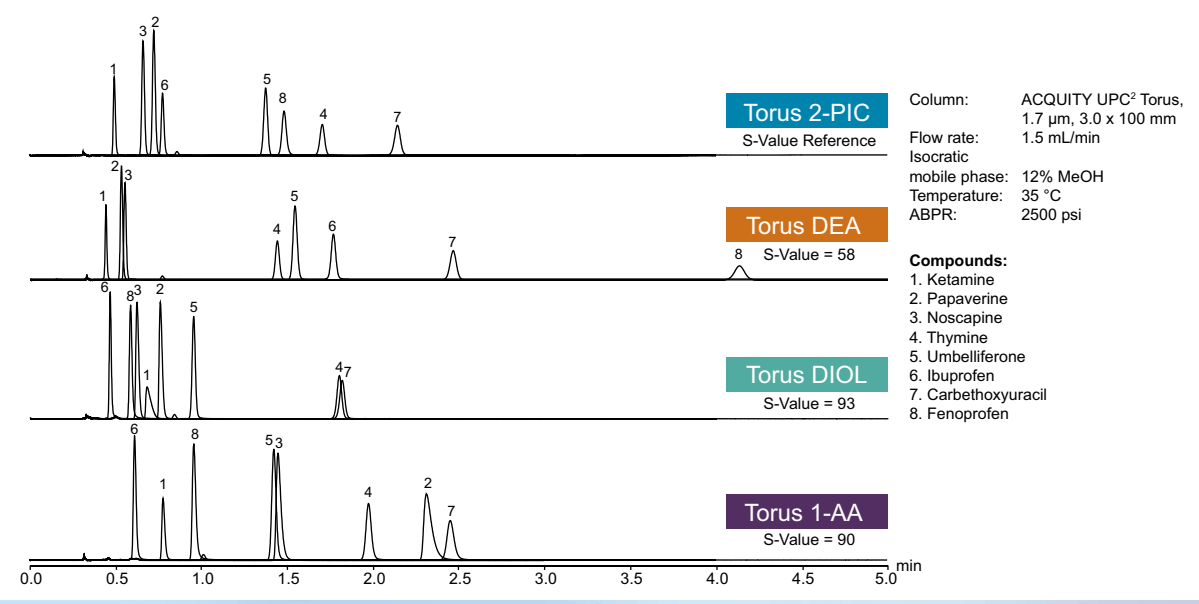
For method development, it is crucial to have a series of columns that have significantly differing selectivities and good retention. The Torus chemistries were specifically chosen to provide a breadth of selectivities for acids, bases, and neutral analytes. The synthesis process has been optimized to yield stationary phases with excellent peak and tailing characteristics, both with and without additives. The development of robust methods requires columns that do not exhibit changes in performance over time (retention or selectivity).

## RECOMMENDED STARTING CONDITIONS FOR ACQUITY UPC² TORUS COLUMNS

| BASIC COMPOUNDS | ACIDIC COMPOUNDS | NEUTRAL COMPOUNDS | MIXTURES or UNKNOWNS |
|---|---|---|---|
| TORUS 2-PIC | TORUS 2-PIC | TORUS 2-PIC | TORUS 2-PIC |
| TORUS DEA | TORUS DIOL | TORUS 1-AA + $NH_3$ | TORUS DIOL |
| | TORUS DIOL + $NH_3$ | TORUS 1-AA + TFA | TORUS DEA |
| | | | TORUS 1-AA + $NH_3$ |
| | | | TORUS 1-AA + TFA |

*Recommended ACQUITY UPC² Torus Column and separation condition steps can help speed up the method development process.*

Selecting the most suitable column and separation conditions can be challenging for the method development scientist. The ACQUITY UPC² Torus Column family is designed to maximize the selectivity choices while providing the optimum peak shape and efficiencies. The four Torus chemistries provide a wide range of selectivities (S-values*) that simplify the method development challenge.




*ACQUITY UPC² Torus family of columns exhibit different selectivities for acid, bases, and neutral compounds.*

* (1) U.D. Neue, E.S. Grumbach, J.R. Mazzeo, K. Tran, and D.M. Wagrowski-Diehl "Method development in reversed-phase chromatography" Chap. 6 in: I.D. Wilson, ed. Bioanalytical Separations, Handbook of Analytical Separations, Vol. 4 Elsevier, Amsterdam (2003); (2) U.D. Neue, J.E. O'Gara, and A. Méndez "Selectivity in reversed-phase separations: influence of the stationary phase" *J. Chromatogr. A* 1127(1–2): 161–174 (2006); (3) U.D. Neue and A. Méndez "Selectivity in reversed-phase separations: general influence of solvent type and mobile phase pH" *J. Sep. Sci.* 30(7): 949–963 (2007).

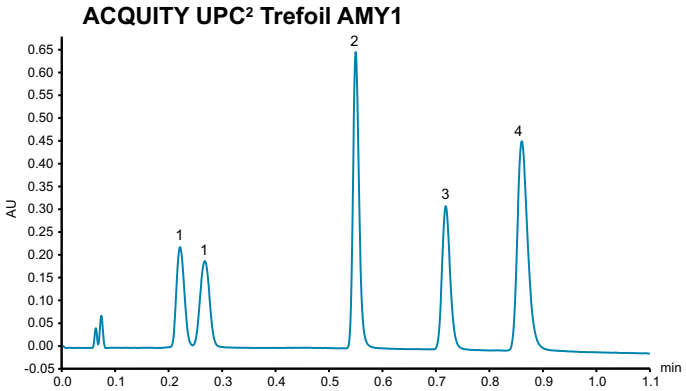
# UPC² Quality Control Reference Material

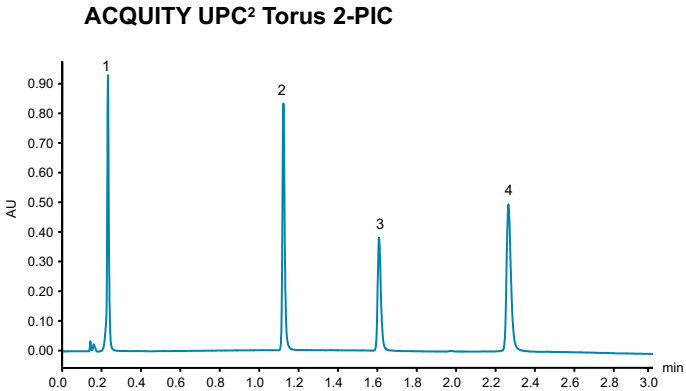
Quality Control (QC) Reference Materials contain mixtures of standards specifically chosen to provide an easy and reliable way to monitor the performance of any chromatographic system. Using QC Reference Materials, you can be assured that your column and system are ready to analyze your samples. Regular use of QC Reference Materials also provides an opportunity to benchmark your chromatographic systems and trend performance over time, making it easier to proactively identify problems and resolve them faster.


**ACQUITY UPC² Quality Control Reference Materials**

| Intended Use | Contents | Part Number |
|---|---|---|
| Provides convergence chromatographic performance information for both chiral and achiral modes. | 1. 0.50 mg/mL (+/-) *trans*-Stilbene oxide<br>2. 0.50 mg/mL Thymine<br>3. 0.50 mg/mL Sulfamethoxazole<br>4. 0.50 mg/mL Sulfamethizole<br><br>In a 1 mL solution of 75:25 ACN:MeOH | 186007950 |

## Single QC Reference Material for ACQUITY UPC² Trefoil and Torus Columns on an ACQUITY UPC² System




**Compounds:**
1. *trans*-Stilbene oxide
2. Thymine
3. Sulfamethoxazole
4. Sulfamethizole

| | |
|---|---|
| System: | ACQUITY UPC² |
| Columns: | AMY1, 2.5 µm, 2.1 x 50 mm<br>2-PIC, 1.7 µm, 3.0 x 50 mm |
| Co-solvent: | Methanol |
| Flow rate: | 2.0 mL/min (3.0 x 50 mm)<br>2.7 mL/min (2.1 x 50 mm) |
| Gradient: | See tables |
| Column temp.: | 50 °C |
| Injection volume: | 2.0 µL |
| Detection (UV): | 240 nm |
| ABPR: | 2,000 psi |

**2.1 x 50 mm Column Gradient**

| Time | %A | %B |
|---|---|---|
| 0.00 | 97 | 3 |
| 1.11 | 40 | 60 |
| 1.39 | 40 | 60 |
| 1.42 | 97 | 3 |
| 2.00 | 97 | 3 |

**3.0 x 50 mm Column Gradient**

| Time | %A | %B |
|---|---|---|
| 0.0 | 97 | 3 |
| 3.0 | 40 | 60 |
| 3.5 | 40 | 60 |
| 3.6 | 97 | 3 |
| 5.0 | 97 | 3 |

*Chromatograms of UPC² QC Reference Material run on ACQUITY UPC² Trefoil and Torus Columns.*

The UPC² QC Reference Material is designed for use with all ACQUITY UPC² Columns. This four-compound mixture was optimized with the following key chromatographic performance factors in mind:

- Compounds are well-separated and cover a wide chromatographic elution range
- Contains a chiral compound to test chiral separation power
- Contains an ionizable compound to test mass spectrometer performance
- All four compounds are compatible with UV detection

*To locate additional information for standards specific to calibration, qualification, and tuning of instruments and detectors, as well as a more comprehensive listing of available standards and reagents, please visit asr.waters.com.*

# Ordering Information

## ACQUITY UPC² Trefoil Columns

| Dimensions | Particle Size | AMY1 | CEL1 | CEL2 |
|---|---|---|---|---|
| 2.1 x 50 mm | 2.5 µm | 186007457 | 186007461 | 186007654 |
| 2.1 x 150 mm | 2.5 µm | 186007458 | 186007462 | 186007655 |
| 3.0 x 50 mm | 2.5 µm | 186007459 | 186007463 | 186007656 |
| 3.0 x 150 mm | 2.5 µm | 186007460 | 186007464 | 186007657 |

## ACQUITY UPC² Trefoil Column Method Development Kits

| Description | Part No. |
|---|---|
| ACQUITY UPC² Trefoil Column Screening Kit, 2.1 x 50 mm columns (AMY1, CEL1, CEL2), 3/pk | 176003577 |
| ACQUITY UPC² Trefoil Column Optimization Kit, 3.0 x 150 mm columns (AMY1, CEL1, CEL2), 3/pk | 176003578 |

## ACQUITY UPC² Trefoil Column Method Validation Kits

| Description | Part No. |
|---|---|
| ACQUITY UPC² Trefoil AMY1 Method Validation Kit, 2.5 µm, 3.0 x 150 mm columns, 3/pk | 186008030 |
| ACQUITY UPC² Trefoil CEL1 Method Validation Kit, 2.5 µm, 3.0 x 150 mm columns, 3/pk | 186008031 |
| ACQUITY UPC² Trefoil CEL2 Method Validation Kit, 2.5 µm, 3.0 x 150 mm columns, 3/pk | 186008032 |

## ACQUITY UPC² Torus Columns

| Dimensions | Particle Size | 2-PIC | DEA | DIOL | 1-AA |
|---|---|---|---|---|---|
| VanGuard™ Pre-Column, 2.1 x 5 mm, 3/pk | 1.7 µm | 186007604 | 186007622 | 186007613 | 186007631 |
| 2.1 x 30 mm | 1.7 µm | 186008109 | | | |
| 2.1 x 50 mm | 1.7 µm | 186007596 | 186007614 | 186007605 | 186007623 |
| 2.1 x 75 mm | 1.7 µm | 186007597 | 186007615 | 186007606 | 186007624 |
| 2.1 x 100 mm | 1.7 µm | 186007598 | 186007616 | 186007607 | 186007625 |
| 2.1 x 150 mm | 1.7 µm | 186007599 | 186007617 | 186007608 | 186007626 |
| 3.0 x 50 mm | 1.7 µm | 186007600 | 186007618 | 186007609 | 186007627 |
| 3.0 x 75 mm | 1.7 µm | 186007601 | 186007619 | 186007610 | 186007628 |
| 3.0 x 100 mm | 1.7 µm | 186007602 | 186007620 | 186007611 | 186007629 |
| 3.0 x 150 mm | 1.7 µm | 186007603 | 186007621 | 186007612 | 186007630 |

## ACQUITY UPC² Torus Column Method Development Kits

| Description | Part No. |
|---|---|
| ACQUITY UPC² Torus Column Screening Kit, 2.1 x 50 mm columns (2-PIC, DEA, DIOL, 1-AA), 4/pk | 176003579 |
| ACQUITY UPC² Torus Column Method Development Kit, 3.0 x 100 mm columns (2-PIC, DEA, DIOL, 1-AA), 4/pk | 176003580 |

## ACQUITY UPC² Torus Column Method Validation Kits

| Description | Part No. |
|---|---|
| ACQUITY UPC² Torus 2-PIC Method Validation Kit, 1.7 µm, 3.0 x 100 mm columns, 3/pk | 186008033 |
| ACQUITY UPC² Torus DEA Method Validation Kit, 1.7 µm, 3.0 x 100 mm columns, 3/pk | 186008034 |
| ACQUITY UPC² Torus DIOL Method Validation Kit, 1.7 µm, 3.0 x 100 mm columns, 3/pk | 186008035 |
| ACQUITY UPC² Torus 1-AA Method Validation Kit, 1.7 µm, 3.0 x 100 mm columns, 3/pk | 186008036 |

## ACQUITY UPC² BEH, CSH, and HSS Columns

| Dimensions | Particle Size | BEH 2-EP | BEH | CSH Fluoro-Phenyl | HSS $C_{18}$ SB, 1.8 µm |
|---|---|---|---|---|---|
| VanGuard Pre-Column, 2.1 x 5 mm, 3/pk | 1.7 µm | 186006575 | 186006557 | 186006566 | 186006616 |
| 2.1 x 50 mm | 1.7 µm | 186006576 | 186006558 | 186006567 | 186006617 |
| 2.1 x 75 mm | 1.7 µm | 186006577 | 186006559 | 186006568 | 186006618 |
| 2.1 x 100 mm | 1.7 µm | 186006578 | 186006560 | 186006569 | 186006619 |
| 2.1 x 150 mm | 1.7 µm | 186006579 | 186006561 | 186006570 | 186006620 |
| 3.0 x 50 mm | 1.7 µm | 186006580 | 186006562 | 186006571 | 186006621 |
| 3.0 x 75 mm | 1.7 µm | 186006581 | 186006563 | 186006572 | 186006622 |
| 3.0 x 100 mm | 1.7 µm | 186006582 | 186006564 | 186006573 | 186006623 |
| 3.0 x 150 mm | 1.7 µm | 186006688 | 186006686 | 186006687 | 186006685 |

| Dimensions | Particle Size | BEH 2-EP | BEH | CSH Fluoro-Phenyl | HSS $C_{18}$ SB |
|---|---|---|---|---|---|
| VanGuard Pre-Column, 2.1 x 5 mm, 3/pk | 3.5 µm | 186006651 | 186006633 | 186006642 | 186006624 |
| 2.1 x 50 mm | 3.5 µm | 186006652 | 186006634 | 186006643 | 186006625 |
| 2.1 x 75 mm | 3.5 µm | 186006653 | 186006635 | 186006644 | 186006626 |
| 2.1 x 100 mm | 3.5 µm | 186006654 | 186006636 | 186006645 | 186006627 |
| 2.1 x 150 mm | 3.5 µm | 186006655 | 186006637 | 186006646 | 186006628 |
| 3.0 x 50 mm | 3.5 µm | 186006656 | 186006638 | 186006647 | 186006629 |
| 3.0 x 75 mm | 3.5 µm | 186006657 | 186006639 | 186006648 | 186006630 |
| 3.0 x 100 mm | 3.5 µm | 186006658 | 186006640 | 186006649 | 186006631 |
| 3.0 x 150 mm | 3.5 µm | 186006659 | 186006641 | 186006650 | 186006632 |

## ACQUITY UPC² Method Development Kit

| Description | Part No. |
|---|---|
| ACQUITY UPC² Method Development Kit, 3.0 x 100 mm (BEH 2-EP, BEH, CSH Fluoro-Phenyl, HSS $C_{18}$ SB), 4/pk | 176003050 |
| ACQUITY UPC² Column Screening Kit, 2.1 x 50 mm (BEH 2-EP, BEH, CSH Fluoro-Phenyl, HSS $C_{18}$ SB), 4/pk | 176003091 |

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Germany 49 6196 400 600

Hong Kong 852 2964 1800

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India 91 080 49292200 03

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May 2016 720004225EN LM-SIG